20. Electrophoresis

Experiment MA-014

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ABSTRACT

A continuous, free-flow electrophoresis study was conducted during the Apollo-Soyuz Test Project mission to investigate and evaluate the increase in sample flow rate and sample resolution achievable in space. This electrophoresis technique is one of the most valuable methods to separate and analyze biological particles, such as living cells, without decreasing their biological activity. Gravity-induced thermal convection and sedimentation limit the effectiveness of separation by this method. The electrophoresis equipment was designed for the separation of four mixtures of biological cells with variable sample flow rates, buffer flow rates, and electric field gradients. Separation quality was assessed by measuring the light from a quartz lamp through the electrophoresis channel and on to a photodiode system.

The data evaluation indicates that all monitored systems operated correctly during the experiment. The optical system produced a light that was too bright to discern true cell distributions, but final analysis of scientific data by computer processing shows the expected distribution of separated cells.

INTRODUCTION

The scientific requirements of the Electrophoresis Experiment (EPE) on the Apollo-Soyuz Test Project (ASTP) flight resulted from a study concerning the application of electrophoresis in future biological experiments on Spacelab. That study examined the possibility of separating vital cells or other biological materials in the space environment.

To understand the specific function of cells and their membrane systems, research is needed concerning the problem of separation and characterization of particles performing individual functions. Of particular importance in studying cooperative interactions in biological processes is the isolation of the interacting systems in the most homogeneous and still functioning form. These problems are not only of a fundamental theoretical interest but are also of practical importance to elucidate normal and pathological processes in biology.

The reason for using electrophoresis to investigate these problems concerns the properties of the cell membrane surface, which is the site of many important biological phenomena. Transformation and differentiation processes are often accompanied by changes of the cell surface charge density. Charge density, however, determines the electrophoretic behavior; i.e., the electrophoretic mobility. Therefore, electrophoresis is a highly efficient method of separating cells or other biological particles according to functional criteria. Furthermore, cell electrophoresis is one of the few physicochemical processes that can be applied to living cells without producing damage or loss of viability.

Because electrophoresis is established as one of the most effective separation methods used in biological study, it is considered an essential tool for investigation in space. The expectation was that the separation capability would be increased

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at zero-g, resulting in new applications that depend on improved resolution.

The EPE concept was based on a new electrophoretic principle known as "carrier-free continuous-deflection electrophoresis" (free-flow electrophoresis). The technique was developed by K. Hannig at the Max Planck Institut and is patented in Germany and the United States. By this method (fig. 20-1), the sample to be separated is introduced continuously into a separation chamber, in which a buffer solution flows laminarly. An electric field is generated perpendicular to the direction of flow. Particles having different surface charge densities are deflected from the flow direction of the buffer by an angle determined by the flow rate and the electrophoretic mobility of the particles. After leaving the lower end of the separation chamber, the separated zones can be collected continuously or analyzed by evaluating the deflection and the density distribution of the fractions.

Because this electrophoresis system is a continuous procedure, it should be applicable for routine use in solving a variety of separation problems in a future Spacelab. The EPE was designed to verify the theoretically expected better and higher capacity separations in the free-flow electrophoresis system under zero-g conditions.

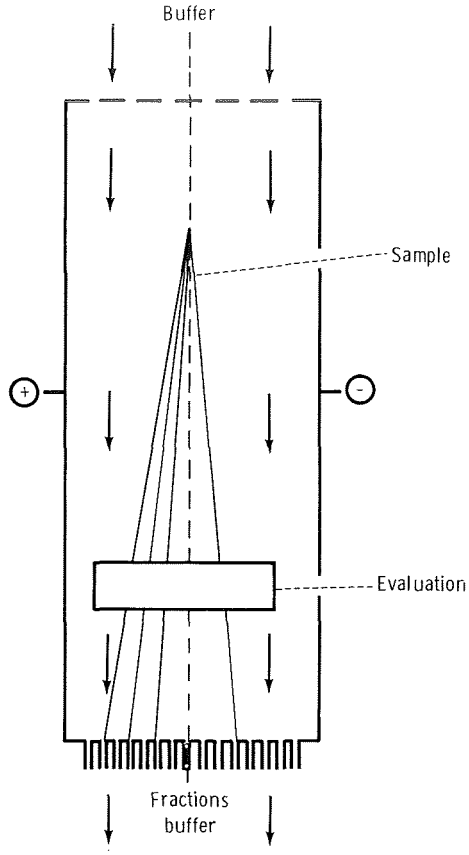

FIGURE 20-1.—Illustration of the principle of free-flow electrophoresis.

EFFICIENCY OF FREE-FLOW ELECTROPHORESIS

The decisive advantages of free-flow electrophoresis result from the principle of continuous operation in a flowing medium. In contrast to most of the usually applied foil- or gel-electrophoresis methods, this method does not accomplish stability by an open-pore carrier medium, but rather permits the medium to flow freely in a narrow gap. To obtain the necessary stability, the capillary forces at the gap walls are used. Under one-g conditions, this method succeeds when the gap width is less than 1.0 mm. This width is also sufficient for an undisturbed electrophoretic migration of larger particles in the electrical field. Unlike the discontinuous electrophoresis method that is stabilized by porous media, the range of application of electrophoresis for preparative purposes can be expanded to include bulk-colloidal mixtures such as suspensions of viruses, bacteria, and cells and their membrane systems. A further advantage of electrophoresis in flowing medium with continuous operation is that the samples to be examined can be quickly exchanged without interrupting the experimental process.

The capability of quickly switching to the optimal experimental conditions combined with a completely automated operation makes this electrophoretic principle applicable to routine analytical separation problems. Thus, it would be possible to separate as many as 100 samples/hr to obtain analytical data concerning the composition of the samples to be examined.

As in every physical process, the effectiveness of free-flow electrophoresis is limited by some methodologically determined disturbances. In the

use of electrophoresis under zero-g conditions, it is necessary to distinguish between effects caused by gravitation (i.e., sedimentation and thermal convection) and effects independent of gravitation. Under zero-g conditions, sedimentation and convection can be eliminated and thus a higher throughput with larger separation chamber gap dimensions can be obtained.

The resolution of electrophoretic separation is, however, hindered by a methodologically caused band broadening, which also exists under zero-g conditions. The band-broadening effects result mainly from (1) the Poiseuille velocity profile of the liquid curtain, (2) the electro-osmotic velocity profile, and (3) the temperature gradient through the liquid curtain thickness; the temperature gradient increases with liquid curtain thickness and electric power (fig. 20-2). The thermal diffusion caused by the extremely short separation times in free-flow electrophoresis can be ruled out.

The extent of the previously mentioned effects on the resulting band broadening has been theoretically and experimentally examined. The results are reported in reference 20-1, from which the following remarks were taken.

> An analytical Free-Flow Electrophoresis apparatus permitted easy observation of all these parameters. It could be shown that the opposing tailing effects of electro-osmosis, and the temperature gradient to the effect of the velocity profiles can be compensated for by appropriate adjustment of the chamber wall zeta potential. (The velocity profile of the liquid curtain flow causes a tailing of the separated bands in the direction of electrophoretic migration, whereas the electro-osmotic velocity profile counteracts this effect.) The experiments showed that there are additional broadening effects due to other not clearly defined parameters. It was observed that the sample inlet geometry plays a considerable role in band broadening. The practical relevance of this observation is that band broadening is reduced if samples are centered in the middle of the liquid curtain. Furthermore, sample bandwidths should be adjusted to values with a starting bandwidth of less than half the liquid curtain thickness. Under such conditions, the bandwidth was reduced so as to approach theoretical values.

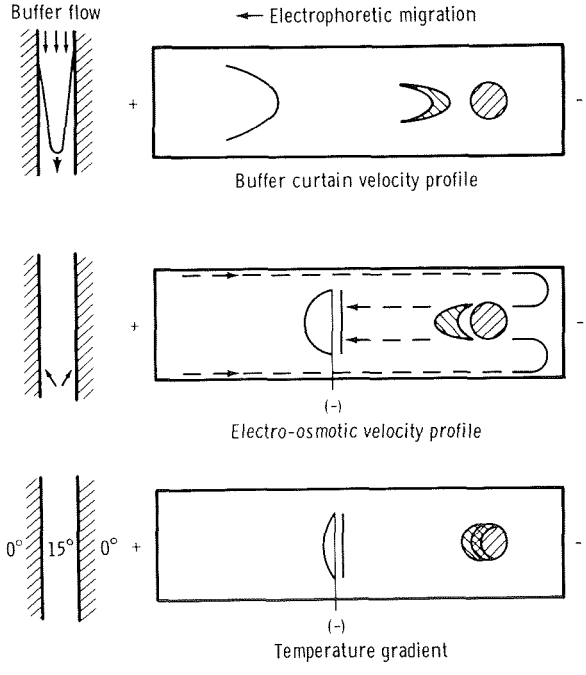

FIGURE 20-2.—Band-broadening effects in free-flow electrophoresis.

These considerations and the experimental results reveal decisive advantanges of free-flow electrophoresis, as opposed to static systems, regarding obtainable separation sharpness and sample throughput. However, one limitation occurs if the method of "isoelectric focusing" or "isotachophoresis" is applied for special separation problems. Because of the discontinuous buffer systems required (pH or ion strength gradients), the free-flow system appears to be less suitable.

ELECTROPHORESIS UNDER ZERO-G CONDITIONS

The efficiency of electrophoresis under one-g conditions is limited. Gravity effects such as heat convection, sedimentation, and buoyancy limit

the application with respect to flow rates and resolution. To avoid convection under one-g conditions, the cross section and dimensions of the electrophoresis separation chamber must be kept small. (The thickness of the buffer film is normally 0.5 to 1 mm.) Under zero-g conditions, there are theoretically no limits in this respect. However, one limit is set by the need for effective removal of the Joule heat, especially when working with biological materials. (Depending on environmental conditions, this limit occurs at a layer thickness of 5 to 10 mm.) Considering this limitation, with a possible tenfold larger buffer film thickness, an increase of up to tenfold in sample throughput can be achieved because of the lack of convection disturbances.

A simultaneous improvement of separation sharpness is, however, not to be expected. As previously discussed, the factors influencing separation sharpness in the EPE are essentially methodological and are not dependent on gravitation. An optimization of separation sharpness can be achieved under one-g conditions if the experimental requirements are fulfilled (suitable separation chamber wall potential and less sample throughput).

The significance of applying electrophoresis in the Spacelab as the preferred separation method for sensitive biological material becomes particularly evident in connection with important biological experiments conducted under zero-g conditions. The use of electrophoresis may assist in clarifying many phenomena, such as growth, metabolism, genetics, and immune responses, which occur differently in zero-g conditions than in one-g conditions. The two main purposes for conducting electrophoresis experiments in space are (1) to analyze purity and to isolate samples for medical and biological research, and (2) to develop separation methods for commercial and other applications on a preparative scale.

Many proposals also merit further investigation. These proposals concern the effect of gravity on cell functions and the ability of cells or whole organisms to adapt to changes in gravity. One example is the cultivation of cells, the investigation of their synthesis efficiency, and their contact inhibition without gravity. Assuming that it is possible to isolate highly purified cell populations grown under zero-g conditions, the state of weightlessness will offer an excellent opportunity to examine the phenomena of cell differentiation and the exchange of information between different cells.

Some of the necessary operational conditions can be tested only with complete absence of sedimentation. The clarification of these phenomena is not only of great scientific interest but also of practical significance for the regulation of detailed biological processes.

The importance of new findings of this kind for medicine can be anticipated. These investigations require a separation method that allows separation of the carriers of specific syntheses or of the synthesis products themselves. This purpose may be fulfilled in Spacelab by using electrophoretic separation methods. However, the applicability of space electrophoresis for the production of biological material for commercial purposes cannot yet be predicted. At this point, researchers are just learning how electrophoresis in space is to be used. The EPE can therefore be essentially regarded as a pilot experiment.

The main purposes of EPE and the problems to be clarified were as follows.

1. Applicability of electrophoresis in space
2. Performance of separation experiments with greater throughput
3. Investigation of temperature and velocity effects with a chamber of larger gap width
4. Investigation of separation capability in zero-g conditions
5. Proof of the possibility of separating preparative quantities of living cells
6. Determination of necessary modifications in the construction of the apparatus for use in space

METHODS AND PROCEDURES

Equipment Design

The design goal for the EPE apparatus (fig. 20-3) was to develop experimental equipment meeting the ASTP spacecraft requirements. The apparatus functions automatically, requiring

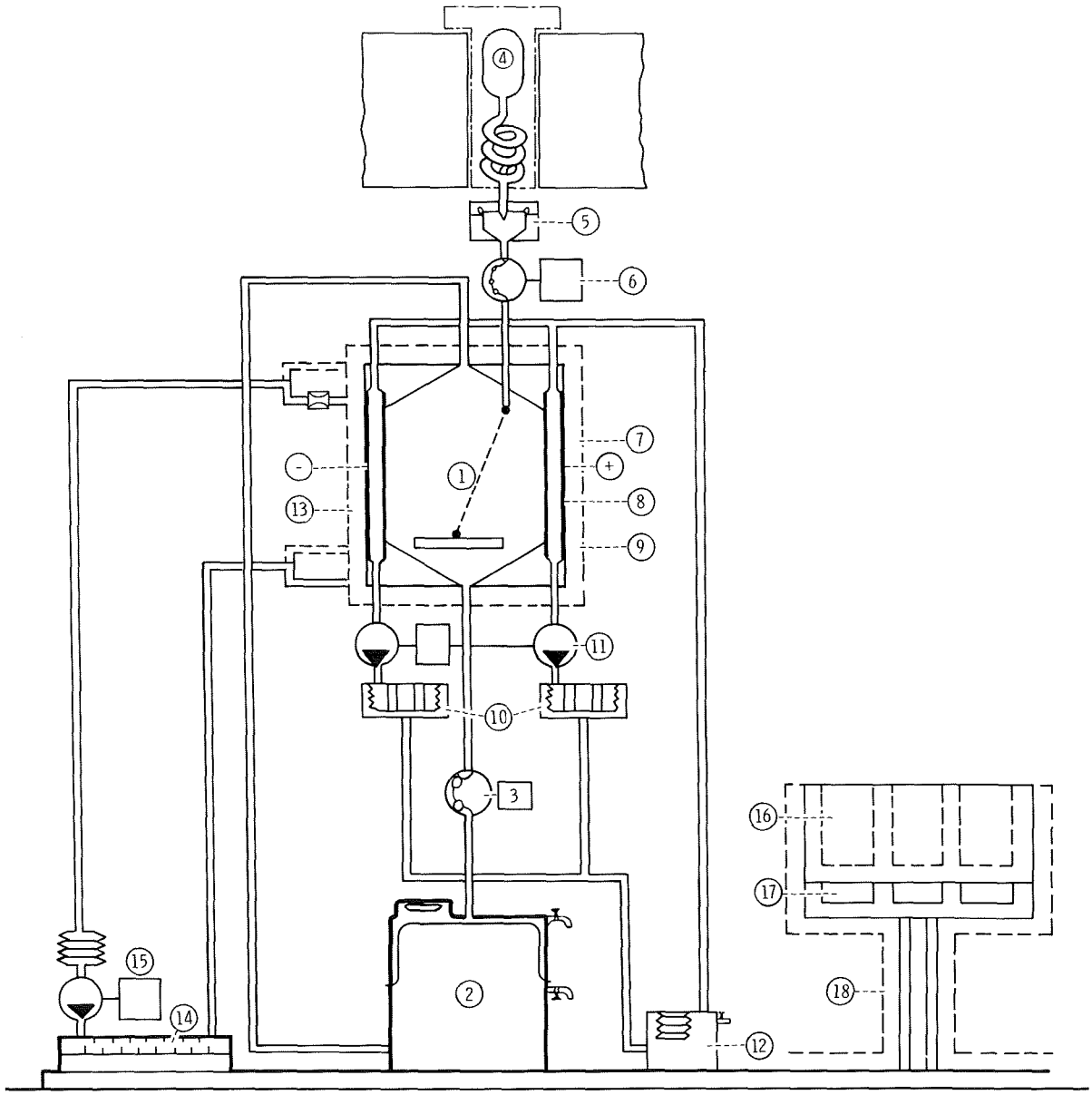

FIGURE 20-3.—Functional diagram of experiment. Buffer system: (1) separation chamber, (2) chamber buffer/waste container separated by diaphragm (3) peristaltic pump and motor for buffer flow. Sample system: (4) sample container (exchangeable), (5) sample inlet, (6) peristaltic pump and motor for sample flow. Electrode system: (7) electrode chamber, (8) electrodes, (9) ion exchange membranes, (10) phase separators ($O_2 + H_2$) and gas absorbers (palladium black), (11) gear pump and motor for electrode purging, (12) electrode buffer container. Chamber cooling system: (13) separation-chamber cooling plates, (14) Peltier heat exchanger, (15) gear pump and motor for coolant flow. Freezer system: (16) sample storage, (17) Peltier cooling unit, (18) heat transfer plate.

minimal crew intervention. Known biological samples were used and it was not necessary to collect the separated biomaterial fractions. An optoelectronic analysis of the separation was performed. A preparative separation was not used. A quantitative evaluation (by use of an optical

system) is sufficient to determine the applicability of the method and to study the sharpness of separation.

The separation chamber is the main part of the apparatus. It consists of two cooling plates that are adjusted to be exactly parallel to supply laminar flow and form a gap of approximately a 28- by 3.8-mm cross section. Along the sides, electrodes (180 mm long) provide the electric field perpendicular to the chamber buffer flow. The electrodes are separated from the chamber buffer by ion-exchange membranes. The electrodes are located in channels purged by the electrode buffer to remove the hydrogen and oxygen (H_2 and O_2) gases generated by the electrolytic processes. The gases are separated from the fluid and absorbed by means of catalysis. The fluid flow is kept constant by controlled pumping at a rate sufficient to transport the total volume of gas generated. The electrode buffer is separated from the cell buffer, which has a lower concentration, by the two ion-exchange diaphragms.

During the experiment sequence, the walls of the separation chamber were cooled down and the temperature was controlled at the operational condition of 278 ±3 K (5° ±3° C). The buffer leaves the storage container and enters the separation chamber somewhat upstream of the sample inlet to achieve a constant laminar flow in the separation chamber parallel to the electrodes. After having passed the separation chamber, the mixture of sample and chamber buffer entered the waste container, which is combined with the storage container but separated by a membrane. The angle of deflection for a particular particle was determined by the separation buffer flow rate and the field gradient. The program included two flow rates for each sample so that the influence of buffer velocity could be studied.

The sample containers were designed so that no air entered the circuit and none of the samples were split when the containers were inserted into the equipment. The containers have a volume of 2 ml, consist of a metal tube with handling knob, and are totally gold plated. On the upper end, they are closed by a pressure exchange membrane and sealing. The lower end has a bore connected to a needle with a valve that is opened by inserting the container into the sample inlet closed by a rubber sealing.

The sample containers were stored in a freezer at 273 to 278 K (0° to 5° C). For experimental operation, they were removed from the freezer and inserted into the equipment so that the sample flow entered the buffer flow. This flow was directed by a peristaltic pump similar to that of the buffer flow circuit.

The separation was evaluated by optoelectronic methods. By this method, a narrow slit across the downstream end of the separation chamber, parallel to the electric field, is illuminated. The light passes through the quartz plates of the separation chamber and is attenuated as a function of the density of the biomaterial distribution. On the other side of the chamber, the light intensity, related to the cell density, is measured with a photodiode array.

Experiment Electronics

The experiment equipment was programed and controlled by electronic units that performed the following functions.

1. Control of the flow rates; i.e., motor speeds for driving the pumps
2. Power supply
3. Program sequencing, which provided the program of the experiment operations
4. Thermoelectric temperature control
5. Self-check and NO-GO indications
6. Data acquisition, evaluation, and processing
7. Data recording
8. Switching and displaying of experiment operations and experiment states

The main purpose of the program sequencer was to operate the experiment automatically as much as possible, thereby reducing crewmember participation. The program sequencer was designed to perform the following functions (fig. 20-4):

1. Timing of functions such as precooling or purging
2. Generating nominal values for voltage and flow rates according to the sequence step

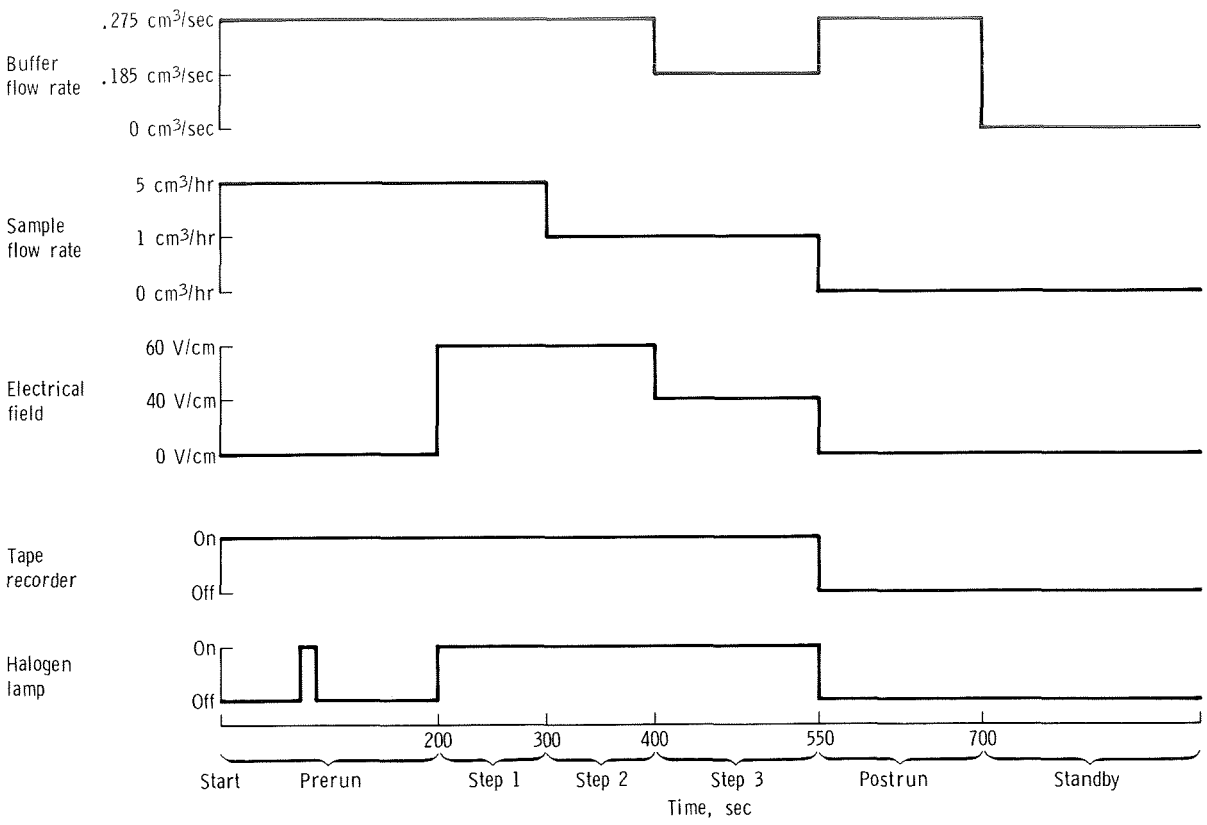

FIGURE 20-4.—Electrophoresis experiment sequence.

3. Collecting and processing GO/NO-GO information from experiment subsystems

4. Switching subunits "on" or "off" according to the program

5. Providing time, frequency, and voltage references

All experimental data were recorded in digital form by two redundant tape recorders that were integrated into the experiment. Both recorded digital data at a bit rate of 2.5 kilobits/sec.

Experiment Hardware

Fluid circuits.—The experiment hardware incorporated four independent fluid circuits.

1. The chamber buffer circuit provides laminar flow for the chamber buffer, which passes from the sample inlet to the quartz window through the separation chamber. The buffer is stored in the chamber buffer container, which at the same time serves to collect the waste chamber buffer leaving the separation chamber. The container halves are separated from one another by a diaphragm that prevents waste buffer from mixing with unused buffer. Thus, the buffer circuit has a constant volume so that there is no need for complicated volume and pressure compensating systems.

2. The sample circuit is used to ensure defined insertion of the biological samples into the separation chamber.

3. The electrode buffer circuit aids in transporting the gases produced at the electrodes to the phase separator where they are separated from the fluid and absorbed by means of catalysis.

4. The coolant circuit is used to keep the temperature of the separation chamber constant (278 K(5° C) wall temperature).

Chamber buffer and sample flow is achieved by

means of peristaltic pumps (figs. 20-5 and 20-6). Coolant and electrode buffer are fed using gear pumps.

Electronics.—The following major problems were solved through the use of electronic equipment (fig. 20-7).

1. Drive and revolutions-per-minute control of motors
2. Control of experiment sequence
3. Temperature control of sample freezer and separation chamber
4. Data acquisition, processing, and storage
5. Automatic functional check of systems and indicators (GO/NO-GO)

Acquisition of scientific data.—The scientific data were obtained through measurement of the absorption of shortwave light passing through the separation chamber to reach the line of measurement (fig. 20-8). After having passed the quartz window of the separation chamber, light from a filtered source is focused onto the optical center of a lens with the aid of a condenser. An image of this line of measurement is projected onto a photodiode array measuring the local brightness of the individual image points.

The photodiode array (fig. 20-9) is composed of 128 photodiodes 12.5 mm in length. Their photovoltages are successively scanned and recorded in digital form on a magnetic tape as a measure of the local brightness. The data rate is approximately 2.5 kilobits/sec with a brightness resolution of approximately 1 percent per step.

Samples.—The equipment was designed for the successive runs of a total of four samples. Each sample had a volume of 1.6 cm^3 usable for analysis. The first three samples were stored at 277 K (4° C) in a sample freezer in the experiment package. The fourth sample was stored in a freezer in the space vehicle and was kept at the temperature of the liquid nitrogen until use. The crew had to insert and exchange the samples; therefore, the containers were designed so that simple handling was ensured, fulfilling at the same time the functional requirements of (1) no entrance of air when inserting the samples into the sample inlet and (2) no escape of fluid when exchanging the samples.

Experiment sequence.—To determine the effects of the individual parameters, the essential parameters influencing the separation were changed in several steps. Each sample was subjected to the same sequence as follows.

1. During the prerun, the sample circuit was refilled with sample fluid and any possible remainders of the previous sample were removed from the separation chamber.
2. During experiment step 1, the following settings were made.

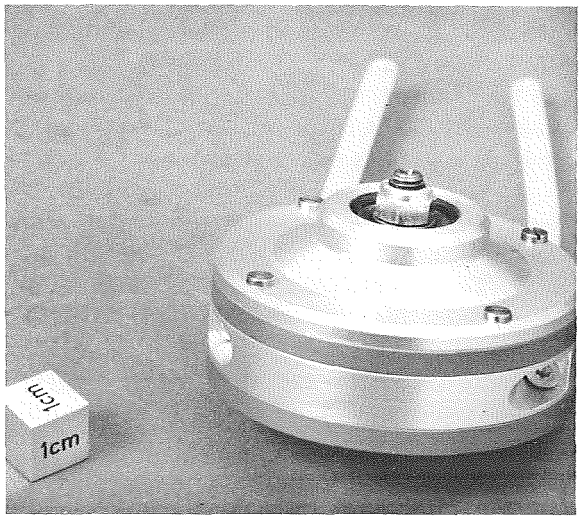

FIGURE 20-5.—Peristaltic pump (weight approximately 150 g) used in chamber buffer circuit and in sample circuit.

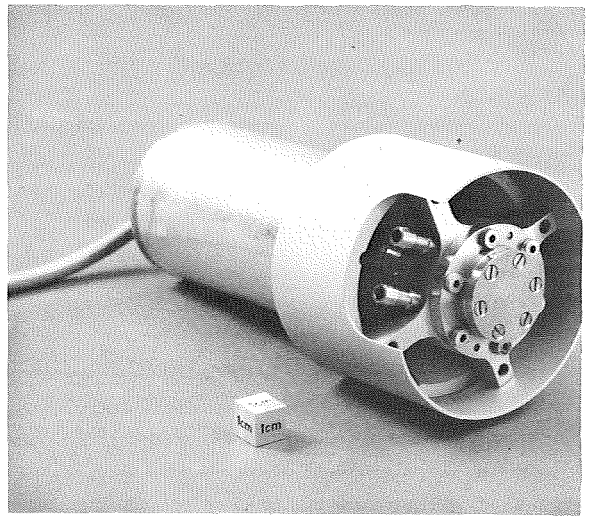

FIGURE 20-6.—Gear pump with motor as used in electrode buffer circuit and in coolant circuit.

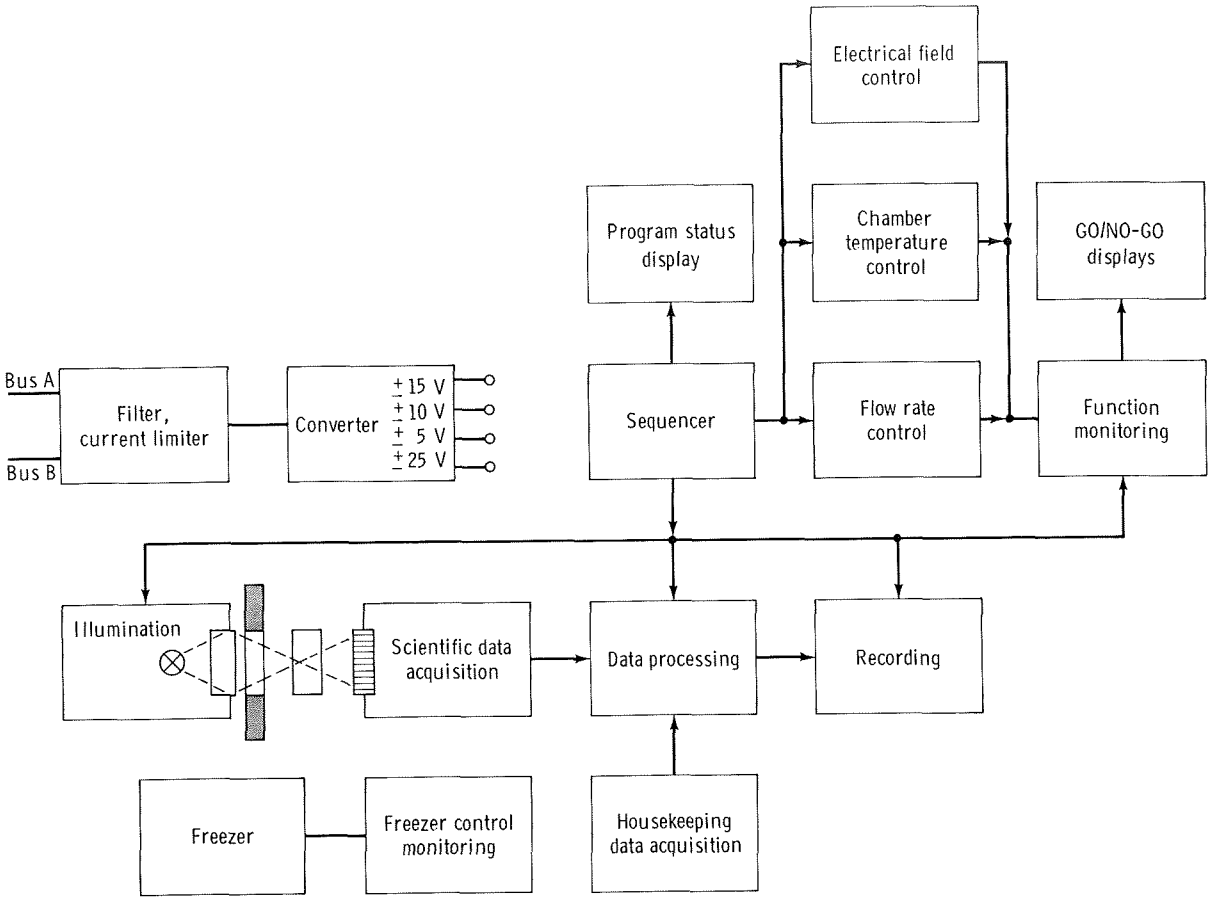

FIGURE 20-7.—Electronics block diagram.

Electric field: 60 V/cm
Buffer flow rate: 0.275 cm^3/sec
Sample flow rate: 5 cm^3/hr

3. During step 2, the sample flow rate was decreased from 5 to 1 cm^3/hr, maintaining the two other conditions.

4. During step 3, the electric field intensity and the buffer flow rate were reduced to two-thirds of their original values, keeping the flow rate constant (1 ml/hr).

The experiment sequence is shown in figure 20-4.

Overall experiment.—The components of the experiment (figs. 20-10 to 20-12) were mounted on a baseplate that also accommodated the heat exchanger and the Peltier elements for control of the chamber temperature.

Special consideration was given to the design to ensure that the equipment was capable of functioning satisfactorily with the cover removed; that all components could be exchanged as easily as possible, and that the major maintenance work, such as sterilizing the fluid circuits and fueling the systems, could be performed with the cover closed.

Cell Sample Preparation

The following samples were prepared for the electrophoresis experiment.

1. Rat bone marrow cells
2. Mixture of human and rabbit erythrocytes
3. Rat spleen cells
4. Rat lymph node cells with the addition of human erythrocytes as markers (Sample 4 was the only frozen sample.)

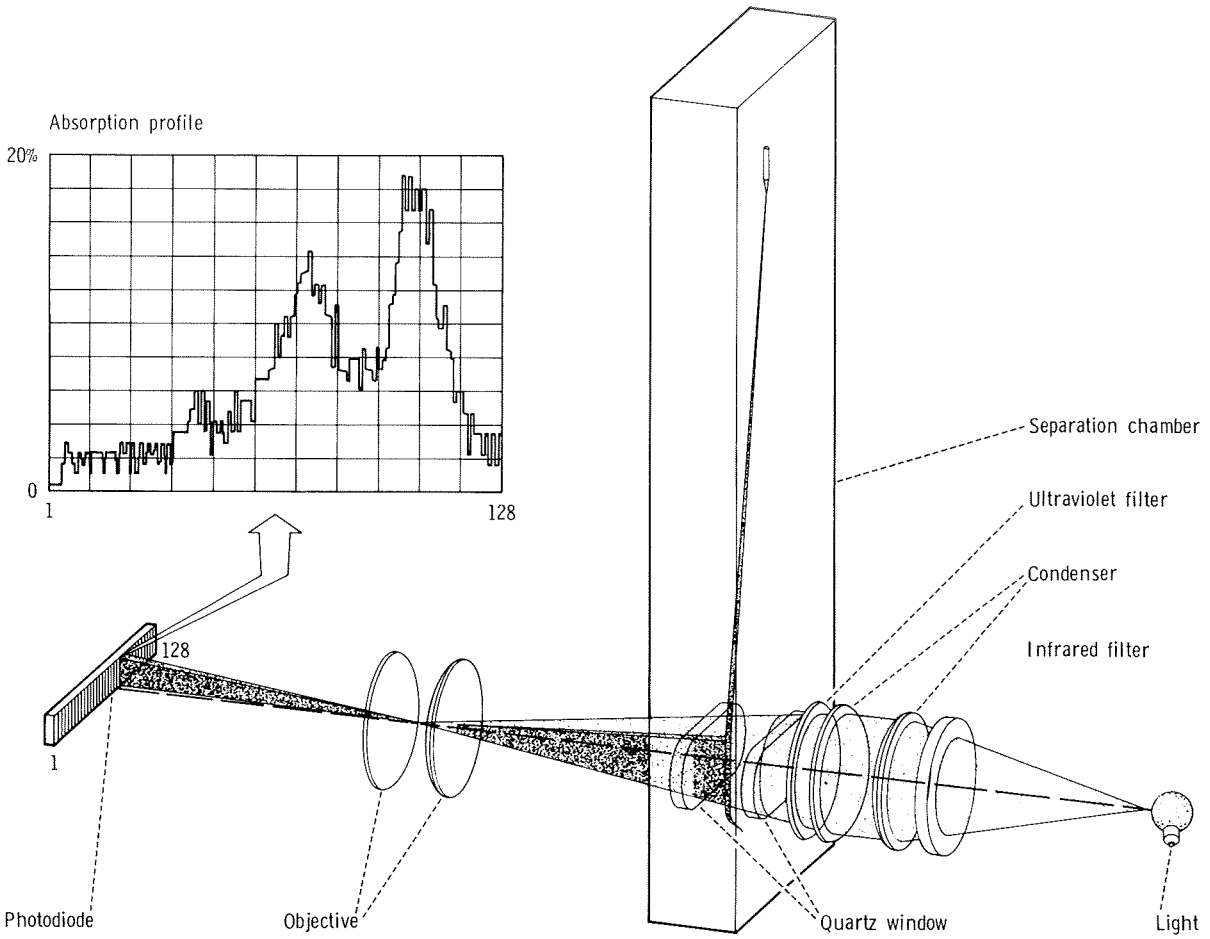

FIGURE 20-8.—Illustration of the principle of sample absorption measurement.

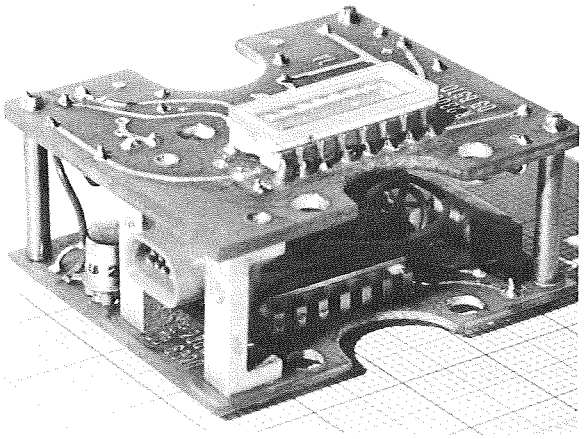

FIGURE 20-9.—Photodiode array integrated into shift register and amplifier unit.

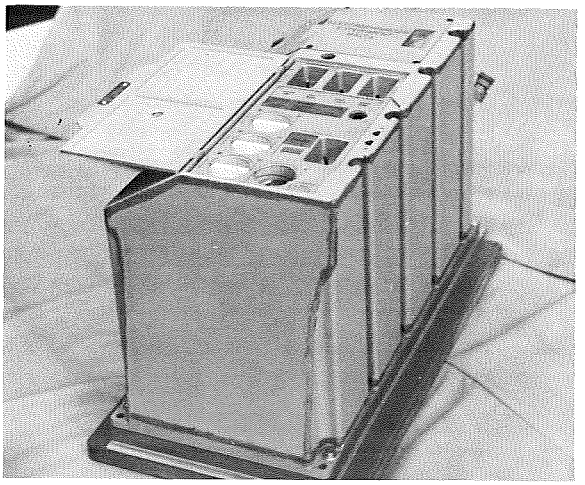

FIGURE 20-10.—Electrophoresis experiment flight unit with protective cover open.

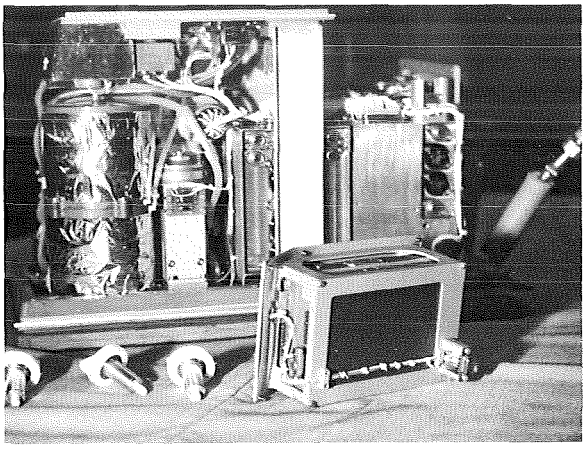

FIGURE 20-11.—Electrophoresis experiment flight unit opened, showing sample container and tape recorder module in the foreground. Components of the experiment shown are the 1.3-liter buffer container (left), the buffer pump with motor and the electrode buffer pump (center), and the electronic components (right).

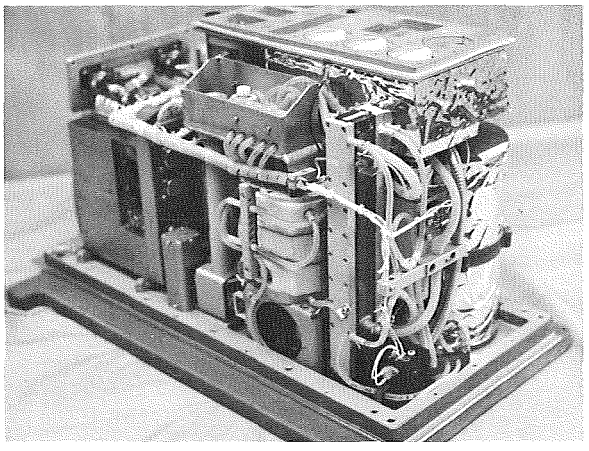

FIGURE 20-12.—Front view of experiment unit. On the right side is the buffer container, and above it the sample freezer and the control panel with sample containers installed. In the foreground is the separation chamber with the hose connections for buffer, coolant, and samples; and below are parts of the optical system. Adjacent to the separation chamber is the electrode buffer container, and above it is the service board for fueling and ventilation. The phase separator is shown below the service board.

With the exception of sample 2 (erythrocytes), all samples were freshly prepared on July 14, 1975, 15 to 20 hours before lift-off. They were stored at 277 K (4° C) for approximately 45 hours before the start of the experiment. Sample 2 (erythrocytes) had been prepared 2 weeks in advance at the Max Planck Institut für Biochemie in Munich, and the cells were preserved by fixation in 2 percent glutaraldehyde. The preparation of cells from different lymphatic organs was accomplished under sterile conditions (autoclaved material, clean bench) at 277 K (4° C) in the NASA John F. Kennedy Space Center (KSC) laboratory.

Bone marrow cells of rats (Wistar) were prepared, and cells were flushed out of the bones with Puck TC solution combined with 2.5 percent bovine serum albumin (33 percent) by using a syringe. A single-cell suspension was prepared by passing the cells several times through a plastic syringe without a needle. The cells were then washed twice (150 g, 10 minutes) with the same buffer and finally resuspended in ampholine buffer.

Cells from spleen and lymph nodes of the rats were prepared by teasing the organs in Puck TC solution at 277 K (4° C) with injection needles. Cells were washed twice with the same solution (150 g, 10 minutes), filtered through cotton wool, and resuspended in ampholine buffer.

For sterile tests, cell suspensions and buffer solution were spread on blood-agar dishes and incubated for 5 hours at 301 K (28° C). This test, as well as light microscopical control, showed no bacterial contamination of cell suspensions and buffer solutions. The viability of the prepared cells (tested with trypan-blue exclusion test) was more than 90 percent in each sample.

All containers of the flight unit were sterilized with formaldehyde (2.5 percent) and washed five times with 20 ml of sterile ampholine buffer. A special apparatus for filling the sample containers was sterilized in the same way. At 4 days before Apollo lift-off, the special "deep freezing container" was filled with a 0.8-ml mixture of rat lymph node cells (25×10^6 cells/ml) and human erythrocytes (30×10^6 cells/ml) and kept in liquid nitrogen. At 14 hours before Apollo lift-off, the other three sample containers were filled with 1.4 ml of the following preparations.

1. A mixture of fixed human erythrocytes

(30×10^6 cells/ml) and fixed rabbit erythrocytes (50×10^6 cells/ml)

2. Native bone marrow cells of the rat (80×10^6 cells/ml)

3. Native spleen cells of the rat (80×10^6 cells/ml)

The exact composition of all buffer systems used for cell preparation and for electrophoretic separation is show in table 20-I.

RESULTS

The experiment began on schedule on July 16, 1975, and lasted for one complete Earth orbit. Radio communication from the Apollo spacecraft confirmed that, according to the signal lamps, all functions were operating properly.

Evaluation of Housekeeping Data

The housekeeping data indicated that the experiment functioned correctly during the mission. The data were examined individually and compared with the values recorded during qualification testing of the equipment (December 1974). In this way, apart from the functional proof, a good evaluation can be made as to the functional reliability of the equipment for a relatively long period. The results of this evaluation are summarized in table 20-II and figure 20-13.

Discussion of Results

Program.—The program sequencer was functioning satisfactorily during all of the four runs. Program sequence and schedule were as planned (fig. 20-13).

Chamber cooling.—The controller used for chamber cooling was functioning properly. During precooling in the prerun, the chamber was cooled to 277.2 K (4.1° C) for each sample, this minimum value being reached toward the end of the prerun. From part I to the end of part II, the temperature control circuit was overloaded during all of the four sample runs. The chamber temperature rose beyond the nominal values of 278 K (5° C) to 280.9 K (7.8° C) (sample 1), 280.6 K (7.5° C) (samples 2 and 3), and 280.3 K (7.2° C) (sample 4), respectively. The maximum value was reached in each case at the end of part II.

In part III, the chamber temperature was again reduced continuously to a final value of 279 K (5.9° C). The circuit was no longer overloaded and

TABLE 20-I.—Buffer Solutions[a]

Description	*Electrode buffer*	*Chamber buffer*	*Ampholine buffer*
Substance, g/liter			
Triethanolamine	5.16	1.71	--
Potassium acetate	0.90	0.30	0.61
Glucose	0.90	14.61	--
Sucrose	--	7.88	--
Calcium acetate	--	0.033	0.05
Magnesium acetate	--	0.46	--
Glycine	--	--	21.55
Ampholine (pH 3.5 to 10)	--	--	20 000
Characteristic			
Conductivity, μmho	2500	900	900
Osmolarity, mOsmol	--	≈300	≈300
pH	7.2	7.2	7.2

[a]Buffer solutions mixed with distilled water.

TABLE 20-II.—Results of Housekeeping Data Evaluation

Item	*Nominal*	*Mission*	*Qualification*
EPE sequence prerun/parts I and II	(a)	(a)	(a)
EPE sequence part III	(a)	(a)	(a)
Reference voltage, V	9.9 to 10.1	9.96 to 9.98	10.00
Chamber temperature, K (°C)	276 to 280 (3 to 7)	277.1 to 280.6 (4 to 7.5)	277 to 279 (4 to 6)
Coldplate temperature, K (°C)	278 to 280 (5 to 7)	278.9 to 279.4 (5.8 to 6.3)	--
Amplifier supply, V	14.2 to 15.8	15.05	15.0
Amplifier supply, V	−14.2 to −15.8	−14.8	−14.82
Logic supply, V	9 to 11	9.8	9.95
Recorder supply, V	4.5 to 5.5	4.75 to 5.0	4.8
Diode array supply, V	24.0 to 25.0	24.7 to 24.8	−25.0
Motor supply, V	20 to 27	20.8 to 23.6	[b]22.5
Electrical field (60 V/cm), V/cm	58 to 62	61 to 61.7	61
Electrical field (40 V/cm), V/cm	39 to 41	40	41
Peltier current, percent	0 to 100	10 to 85	10 to 85
Lamp supply	--	--	--
Reference voltage, V	9.9 to 10.1	10.00 to 10.01	−10.01
Reference voltage, V	9.9 to 10.1	10.0	−10.00
Reference voltage, V	9.9 to 10.1	10.02 to 10.026	9.99
Reference voltage, V	9.9 to 10.1	10.00	10.00
Display supply, V	4.5 to 5.5	4.70	5.05
Auxiliary motor supply, V	20 to 28	22 to 26	[b]24.2
Speed motor 2	(a)	(a)	(a)
Speed motor 1	(a)	(a)	(a)
GO/NO-GO events	(a)	(a)	(a)

[a] Satisfactory.
[b] KSC test, Oct. 1, 1975.

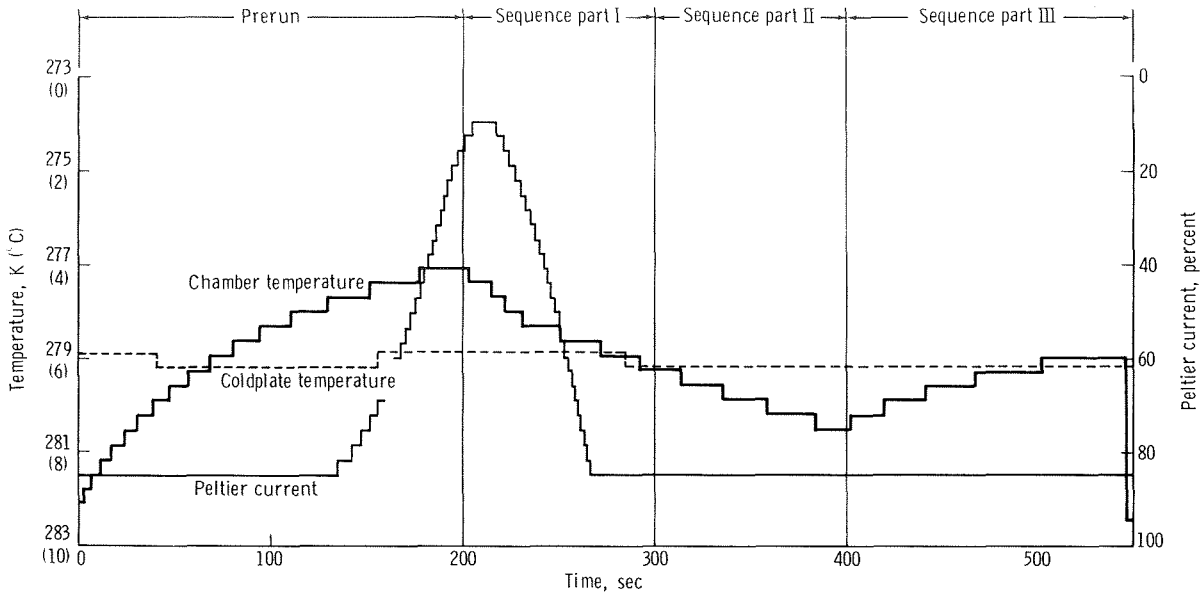

FIGURE 20-13.—Temperature profiles of the experiment during the mission (applicable for all four samples).

reached its nominal temperature, as shown by the extrapolation. The latter behavior, which differs from that in part I and part II, was due to the more than 50 percent reduction in heat transformation during part III of the sequence.

During the testing, an improvement in the temperature behavior was recognizable, which, as experience has shown, results from desalting of the electrode buffer and, thus, a decrease in the conductivity of this buffer as a function of operating time.

Overloading of the temperature control circuit during parts I and II of the sequences resulted from the following.

1. The conductivity of the chamber buffer was set to 800 μmho/cm at a temperature of 283 K (10° C) for the mission. Desalting of the electrode buffer, in principle, results in approximately a 10-percent increase in the conductivity of the chamber buffer in the separation chamber so that a mission conductivity of approximately 880 μmho/cm was obtained. Compared with the nominal value (750 μmho/cm), a current increase of 18 percent, or a power increase of approximately 35 percent, was achieved.

2. Upon completion of the hardware design, the conductivity of the electrode buffer was increased to 2500 μmho so that the electrode buffer cross section no longer affected the overall resistance of the separation chamber. This led to another current increase of approximately 10 percent, compared to the nominal value (20 percent power increase).

3. The modification of the cooling pump brought about a 30-percent reduction in coolant flow rate so that the stability reserve preventing the coolant circuit from overloading was depleted.

The assessments, based on the performance parameters thus modified, have shown that the cooling system behavior must have been as shown during the mission. As indicated in table 20-III, the temperatures as a whole did not exceed the prescribed performance limits.

Electric fields.—The prescribed field voltages in both ranges (i.e., 60 and 40 V/cm were maintained with a high degree of accuracy during all four sample runs (table 20-II).

Supply voltages.—The supply voltages were within the prescribed tolerance range; minor deviations of the mission data from the comparison values obtained during qualification testing are irrelevant.

Illumination.—As a result of the weightlessness, the circuit process in the halogen lamp was disturbed so that the helix temperature during the mission was higher than that under one-g condi-

TABLE 20-III.—Temperature Behavior of Experiment

Sample no.	Temperature, K (°C)			
	Chamber (initial)	Minimum (end of prerun)	Maximum (end of part II)	Minimum (end of part III)
1	282.5 (9.4)	277.2 (4.1)	280.9 (7.8)	279 (5.9)
2	282.2 (9.1)	277.2 (4.1)	280.6 (7.5)	279 (5.9)
3	282.2 (9.1)	277.2 (4.1)	280.6 (7.5)	279 (5.9)
4	282.5 (9.4)	277.2 (4.1)	280.3 (7.2)	279 (5.9)

tions. To obtain information about the excessive lighting, the transient phenomena of the illumination unit were determined at the beginning of each lamp check performed before each mission sequence, and the final lighting value was defined through extrapolation.

The transient phenomena of the illumination unit are shown in figure 20-14. Assuming that the transient follows the exponential law, the following equation applies approximately.

$$y = y_\infty(1 - T\dot{y}) \tag{20-1}$$

where y is brightness, $\dot{y}$ is its derivative, and T is time. Time constant T_1 is then given by

$$T_1 = \frac{1}{\dot{y}_1}\left(1 - \frac{y_1}{y_{1\infty}}\right) \tag{20-2}$$

Assuming that T_1 of the curve for normal lighting and T_2 of the curve for excessive lighting are approximately equal, a conditional equation is obtained for the final value $y_{2\infty}$ from the curve for excessive lighting

$$y_{2\infty} = \frac{y_2}{1 - \left(1 - \frac{y_1}{y_{1\infty}}\right)\frac{\dot{y}_2}{\dot{y}_1}} \tag{20-3}$$

Using the values shown in figure 20-14, then $y_1 = y_2 = 0.7$ (lower end of measuring range), $\dot{y}_1 = 1.677 \times 10^{-3}$ sec^{-1} (with $y_1 = 0.7$), $\dot{y}_2 = 2.5 \times 10^{-3}$ to 2.85×10^{-3} sec^{-1} (with $y_2 = 0.7$), and $y_{1\infty} = 0.95$ (final value of normal curve). The probable final value of the photodiode array subjected to excessive lighting is given by $1.18 \leq y_{2\infty} \leq 1.27$.

Reference voltages.—The reference voltages registered on channels 136 and 147 to 150 are decisive for the reproducibility of the data. The deviations of the reference voltages from the values obtained during qualification testing show the level of confidence of the calibration curves. None of the voltages revealed a deviation larger than 3.6 percent.

Lamp voltage.—After a modification of the illumination system performed on a short-term

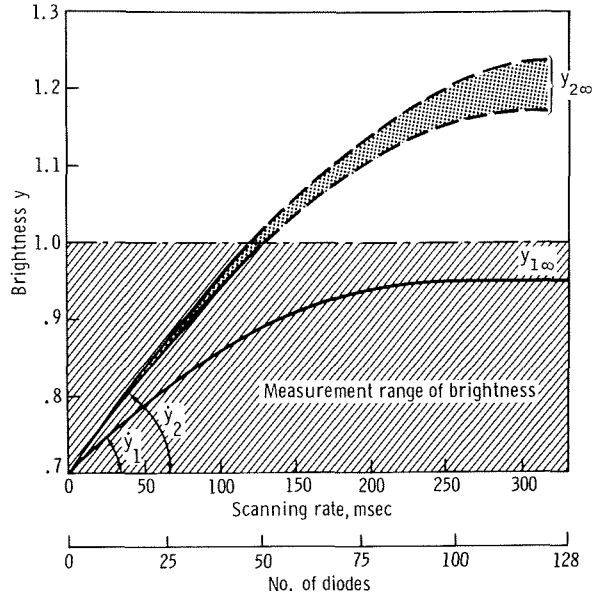

FIGURE 20-14.—Transient behavior of the illumination system.

basis, adaptation of the brightness sensor from 40 to 10 W was abandoned because of cost and schedule reasons; therefore, no data were supplied by this channel.

Motor speeds of peristaltic pumps.—The motor speeds were within the prescribed tolerance range. Stepper motors were used; therefore, only two stable states are possible, which means that the motors either run at nominal speed or they do not run at all. In addition, the NO-GO indicators and the event combination (in channel 161) confirmed that the motors were operating correctly.

NO-GO and status checks.—No NO-GO was recorded throughout the mission. The experiment status was displayed as requested by the program.

Interfaces.—The coldplate temperature ranged between 279 and 279.3 K (5.9° and 6.2° C) throughout the mission. The terminal voltages of bus A and bus B were within the prescribed tolerance range, as derived from the evaluation of channels 142 and 152 (uncontrolled voltages).

Tape recordings.—The tape recordings could be read correctly by the unit tester. The synchronization and bit errors were smaller than 1 percent. Of the total of 7500 frames, approximately 50 could not be read. The errors essentially were due to data failures during starting and stopping of the tape recorder at the beginning and end of the sequences (four samples and preinstallation test).

Evaluation of Scientific Data

The scientific data revealed that the light for the optical detection of the separation must have been too bright. The lamp check showed excessive lighting that resulted in a saturation of the photodiodes in the optical detection system. The quantitative estimate of error revealed that the light must have been 35 percent too bright, although the lamp check indicated normal functioning during the preinstallation check. For this reason, only absorptions above 25 percent were registered. This amount corresponds to the maximum expected absorption of the separated fraction zone.

In the entire separation process, no "true" cell distribution curve was obtained. The baseline of registration in all cases was above the maximum absorption obtained in the peaks. Because of this finding, an analysis of the experiment seemed impossible at first. However, during the stationary phase of the separation (after adjustment to constant experimental conditions), i.e., during 90-second separation time, irregularly occurring pulses (called "events") were recorded. This pattern reflected the expected course of the separation curves (fig. 20-15). These pulses seemed to be caused by cell aggregation in the region of the separated bands. This assumption was confirmed in the following two ways.

1. An aliquot of the sample used in the ASTP flight was separated under the same conditions on lift-off day at KSC, in an identical apparatus but under one-g conditions (electrophoresis engineering model (EM) with a 0.7-mm buffer film thickness). In addition to the expected cell distribution curves (fig. 20-15), analysis of this undisturbed tape showed stray pulses caused by cell aggregation that had a greater absorption. The accumulation at the same location (frequency distribution of the events) during a separation time of approximately 2 minutes yielded the distribution curves (fig. 20-15).

2. To check this finding and to obtain a reliable interpretation, such separations were repeated many times with the EM apparatus, and each experiment was performed and evaluated with normal and increased lighting. It was clearly established that only the analysis of the periodically occurring cell aggregations at the measuring location was representative of the actual cell distribution. The difficult evaluation and coordination of indirect information was performed with the aid of a computer.

Based on these studies, assertions can be made regarding the following samples.

1. Bone marrow cells: Although only a few events (73) could be analyzed because of the tendency of these cells to form no aggregations, the distribution curve is that of an excellent separation.

2. Mixture of human and rabbit erythrocytes: Because of the weak tendency of these fixed cells to form aggregations, insufficient information was available for computation.

3. Spleen cells: The best result was obtained with this sample. The amount of information (358 events) was sufficient to identify fine details of

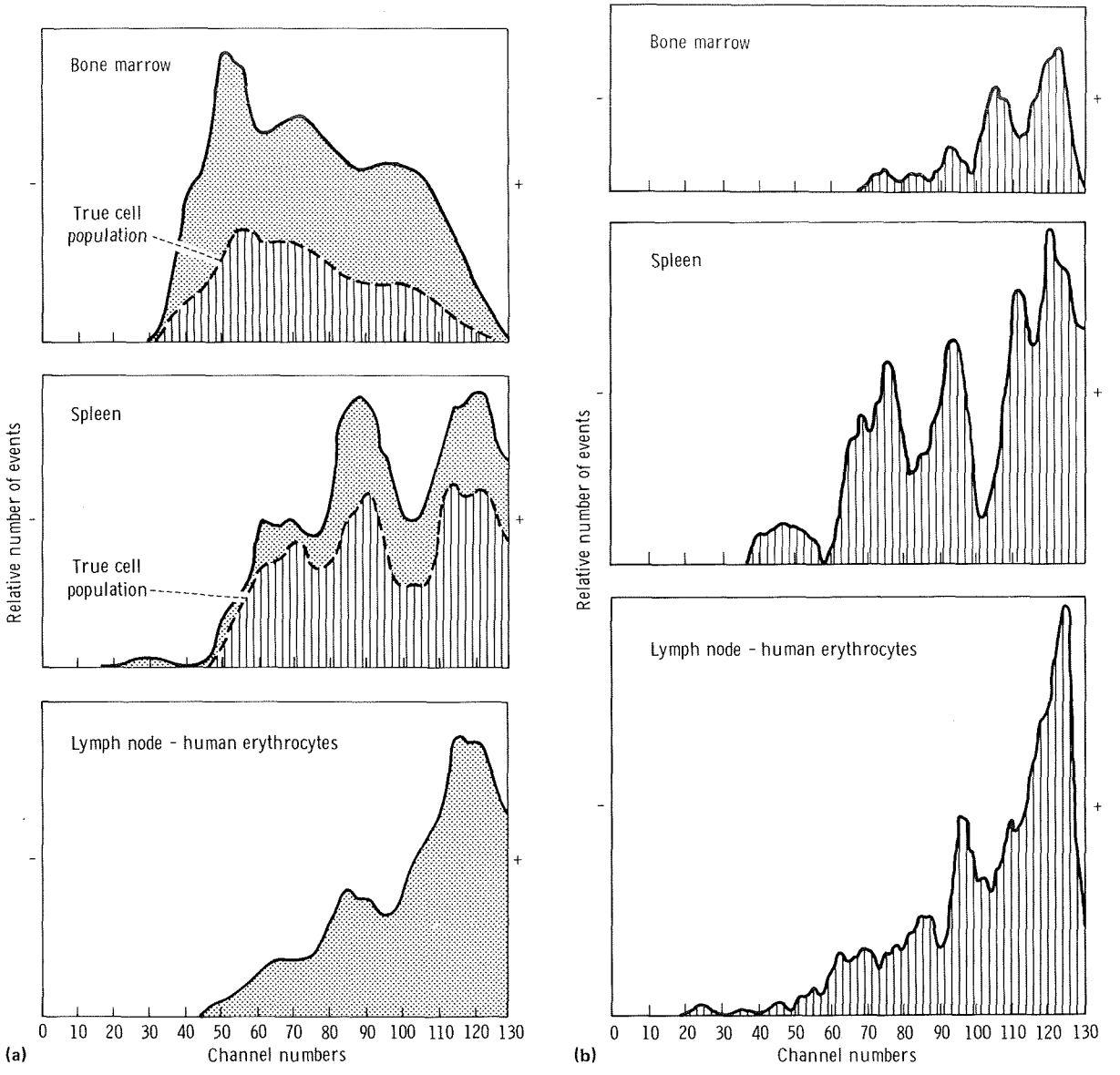
Bone marrow
True cell population
Spleen
True cell population
Lymph node - human erythrocytes
Relative number of events
0 10 20 30 40 50 60 70 80 90 100 110 120 130
Channel numbers
(a)
Bone marrow
Spleen
Lymph node - human erythrocytes
Relative number of events
0 10 20 30 40 50 60 70 80 90 100 110 120 130
Channel numbers
(b)

FIGURE 20-15.—Sample separation under zero-g and one-g conditions. (a) zero-g conditions. (b) One-g conditions.

the separation. The separation in erythrocyte peak can sometimes be indicated in good separations under normal one-g conditions as well. This finding indicates an essentially good separation sharpness under zero-g conditions.

4. Lymph node cells with the addition of human erythrocytes (frozen sample): A large number of events (549) were detected. A good separation effect, similar to sample 3, was inferred here as well as under zero-g conditions.

Postflight Examination

Postflight examination of the flight unit, which took place in Munich, yielded the following information.

1. All housekeeping data were within the tolerance range.

2. The buffer and sample amounts corresponded to the required specification according to the volume measurement carried out.

3. Buffer samples collected in the container revealed no extraordinary bacterial contamination despite the long storage period. Microscopic investigation for the presence of cell particles in the separation buffer (collection container) was positive.

4. The expected remainder of the cell suspension, more than 3 months old, was found in the examined sample containers. However, the gold-plated containers revealed unexpected corrosion.

5. All buffer tube systems were intact and penetrable. The pump systems were still fully operational.

This information clearly reveals that the course of buffer and sample and the electronic system must have operated accurately. Surprisingly, the error in the optical detection system shown by the tape (saturation of the photodiodes) was at first not identifiable. Both the current and the voltage of the lamp were normal. By contrast, the light intensity measured by the diode array was, as expected, too low, because of turbidity. The cause of this phenomenon can be considered as either a fault in the electrical contact that occurred during the flight or an increase in lamp helix temperature that occurred under zero-g conditions. The latter could be explained by the absence of thermal convection in the gas-filled tube.

SUMMARY

The applicability of free-flow electrophoresis under zero-g conditions of the space flight was confirmed. The technical problems arising from the special environmental conditions in a Spacelab can be controlled. This observation applies to the technical concept of the electrophoresis experiment apparatus as well as the manual operation by crewmembers. In particular, it was demonstrated that the buffer flow systems operated despite the more difficult conditions imposed by a closed system. The effective removal of gases from the electrode buffer (electrolytic products) by the measures used was a necessary part of this experiment.

In a separation chamber of large cross section, the temperature conditions required for biological material could be fulfilled. This also applies for the correct laminar buffer flow.

A sample throughput greater than tenfold was achieved by the expansion of the separation chamber cross section, possible in space flight, and with otherwise similar chamber dimensions. (In the fast sequence (fig. 20-4, step 1), 7×10^6 cells/min were separated.) Despite the error that occurred in the optical detection system, it was demonstrated that the separation sharpness corresponded at least to that of analytical separations under one-g conditions. Whether an improved separation sharpness is possible with a fivefold decreased sample insertion rate (fig. 20-4, steps 2 and 3), in contrast to one-g conditions, could not be determined because of the reduced source of information (sensitivity) caused by the lamp error. This is of little importance because such measurements can also be conducted under one-g conditions, as already shown. Also, the separation sharpness depends on other influences as well (e.g., zeta potential on the wall).

The possibility of separating living cells under zero-g conditions was demonstrated. The cell aggregations that formed in the cell suspensions need not correspond to a decrease in cell viability.

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